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Evolutionary radiation of present-day *Nautilus* and *Allonautilus*

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Abstract: The extensive fossil record of coiled nautiloids indicates that they comprised a diverse assemblage of species in ancient oceans. Today they are represented by the genera *Nautilus* Linnaeus, 1758 and *Allonautilus* Ward and Saunders, 1997, inhabiting reef systems throughout the Indo-Pacific region. Some individual populations of *Nautilus* show subtle differences in shell morphologies, and these morphological differences may be used to diagnose different species. An alternative view is that these differences are simply geographically localized, morphological variants within the broadly distributed taxon generally referred to as *Nautilus pompilius* Linnaeus, 1758. Here we present a hypothesis for the phylogeny of present-day *Nautilus* and *Allonautilus* using molecular characters from two mitochondrial gene regions, 16S rDNA and Cytochrome Oxidase *c* subunit I. Populations of *N. pompilius* in Indonesia (Ambon Strait), the Philippines, Vanuatu (New Hebrides Islands), Papua New Guinea, Carter Reef and Osprey Reef (Great Barrier Reef, Queensland, Australia) and *N. macromphalus* (Sowerby, 1849) in New Caledonia were surveyed as well as samples of *N. repertus* (Sowerby, 1849) (Rowley Shoals, Western Australia), *N. belauensis* (Saunders, 1981) (Palau), and *N. stenomphalus* (Sowerby, 1849) (Queensland, Australia). The gastropod *Crepidula striolata* (Menke, 1851) was included as an outgroup. Our results indicate that *Nautilus* is currently undergoing a period of evolutionary radiation throughout the Indo-Pacific region. The topology of the strict consensus tree suggests that the basal divergence between *Nautilus* and *Allonautilus* occurred in the waters surrounding the present-day island of New Guinea and the northern part of the Great Barrier Reef in northeast Australia. This was followed by a migration to the east by the common ancestor of *N. macromphalus* and the *N. pompilius* populations in Vanuatu, Fiji, and American Samoa. A subsequent migration to the west led to the founding of populations off the west coast of Australia, the Philippines, Palau, and Indonesia. Our results also indicate that *N. macromphalus* and *A. scrobiculatus* are phylogenetic species. However, *N. pompilius* is a paraphyletic assemblage of populations and does not represent a true phylogenetic species. Divergences within the genus *Nautilus* appear to be driven by geographic isolation, and we discuss how this may be a result of constraints on dispersal imposed by the ecology of the animals.

Key words: cephalopod phylogeny, speciation, phylogeography

During most of the Phanerozoic, the world's oceans were inhabited by a vast array of externally shelled cephalopods. Today, fewer than a dozen species survive. These animals, comprising the genera *Nautilus* Linnaeus, 1758 and *Allonautilus* Ward and Saunders, 1997 are of considerable interest to both evolutionary biologists and paleontologists because they serve as model organisms for understanding the general biology of extinct nautiloids. A survey of the fossil record indicates that nautilids, the restricted group containing *Nautilus* and *Allonautilus*, experienced a series of radiations and extinctions throughout their history (Ward 1988). Although nautiloids are well represented in the fossil record prior to the Miocene, the more recent fossil record contains few representatives (Eocene–Saunders *et al.* 1996; Miocene–Teichert and Matsumoto 1987; Lower Pleistocene–Wani *et al.* 2007). The rarity in the fossil record during the past 20 million years, of a once abundant lineage of nautilids persisting to the present day suggests that populations of nautilids must have been small during this period. This is also suggested by low levels of genetic divergence in living nautilids (Woodruff *et al.* 1987, Wray *et al.* 1995, Bonnaud *et al.* 2004).

Although the fossil record of nautilids has been explored in detail (Miller 1947, Kummel 1956, Matsumoto 1983, Tintant and Kabamba 1983), little is known about the relationships of the extant forms, and the actual number of species and genera is a matter of debate. Eleven species have been proposed, and at least seven are thought to be synonymous: *Nautilus pompilius* Linnaeus, 1758 (= *N. ambiguus* Sowerby, 1849 = *N. alumnus* Iredale, 1944); *N. scrobiculatus* Lightfoot, 1786 (= *N. umbilicatus* Lister, 1685 = *N. perforatus* Conrad, 1849 = *N. texturatus* Gould, 1857); *N. macromphalus* Sowerby, 1849; *N. stenomphalus* Sowerby, 1849; *N. repertus* Iredale, 1944; and *N. belauensis* Saunders, 1981. Saunders (1987) concluded that there are only four recognizable species: *N. pompilius, N. macromphalus, N. scrobiculatus,* and *N. belauensis.* He regarded the status of *N. repertus* and *N. stenomphalus* as questionable. Ward and Saunders (1997) proposed the new genus *Allonautilus* to

group together *N. scrobiculatus* and *N. perforatus*, based on the extreme morphological differences of these species relative to all other extant species, as well as the levels of genetic divergence reported by Wray *et al.* (1995) and Woodruff *et al.* (1987). The validity of this genus has been subject to debate (Harvey *et al.* 1999, Ward 1999). We provisionally adopt the usage of Ward and Saunders (1997). To avoid confusion, we refer to the animals in both genera as nautilus with a lowercase "n" and non-italicized. When we refer to the genus *Nautilus*, we use uppercase and italics.

Part of the taxonomic confusion regarding nautilus results from the fact that all of the proposed species, with the exception of *Nautilus belauensis*, were originally described from drift shells collected on beaches throughout the Indo-Pacific (Fig. 1). Specimens that possessed unique shell morphologies were identified as new species, but the degree of morphological variation within populations was unknown. Because nautilus shells can be transported great distances by surface currents, making it virtually impossible to ascertain their point of origin (Saunders and Spinosa 1979, House 1987), it was difficult to assess whether morphological differences in shells used for species diagnoses were fixed within populations, making them appropriate diagnostic characters (*sensu* Davis and Nixon 1992), or variation within a single species with a broad geographic distribution.

Figure 1. Specimens of the shells of various putative species of Nautilus. A, *Nautilus macromphalus* Sowerby, 1849, New Caledonia (AMNH 3078570). B, *Nautilus belauensis* Saunders, 1981a, Palau (AMNH 43027). C, *Nautilus stenomphalus* Sowerby, 1849, Carter Reef, Australia (AMNH 43260). D, *Nautilus pompilius* Linnaeus, 1758, Papua New Guinea (AMNH 43028). E, *Nautilus repertus* Iredale, 1944, northwest Australia (AMNH 58545). F, *Allonautilus scrobiculatus* Lightfoot, 1786, Papua New Guinea (AMNH 43261).

The first information regarding the distribution of the various species that had been proposed can be found in the work of Arthur Willey, who trapped specimens in and around New Guinea and New Caledonia from 1894 to 1897 (Willey 1899, 1902). Willey's results, along with those of subsequent expeditions in the 1970s and 1980s indicated that four species previously proposed based on shell morphology (*Nautilus macromphalus, N. belauensis, N. stenomphalus,* and *Allonautilus scrobiculatus*) were localized to particular reef systems (Saunders 1981a,1981b, Saunders and Ward 1987a, 1987b, Ward 1988). *Nautilus macromphalus* occurs in New Caledonia and the Loyalty Islands, *N. belauensis* on reefs near Palau, *N. stenomphalus* off the coast of Queensland near the Great Barrier Reef in Australia, and *Allonautilus scrobiculatus* in Papua New Guinea. In contrast, *N. pompilius*, the type species for the genus, appears to have a much broader distribution. The documented western limit of its range is the

Andaman Islands of Indonesia, although individuals have been collected as far west as the Seychelles and Mauritius in the Indian Ocean. The eastern extreme is American Samoa. To the south, drift specimens have been collected in New Zealand and on the coast of New South Wales in Australia. Populations of *N. pompilius* occur throughout the Philippines; these appear to represent the northernmost limit of the distribution of present-day nautilus (Saunders 1987).

Wray *et al.* (1995) performed a phylogenetic analysis of *Nautilus* and *Allonautilus* using 10 morphological characters and mitochondrial and nuclear DNA sequences. Their results indicated low levels of sequence divergence, and they recovered only two phylogenetic species (*sensu* Cracraft 1983), *A. scrobiculatus* and *N. pompilius*. The *A. scrobiculatus* samples formed a monophyletic group that was the sister group to the genus *Nautilus*. *Nautilus* in turn contained three clades that corresponded to the biogeographic distributions of the populations and species included in the study, which the authors designated the Western Pacific, Australia/Papua New Guinea, and Western Australia/Indonesia clades. Each clade contained representatives of different populations of *N. pompilius* collected in each of these areas, as well as a representative of one of the other proposed species collected in the same geographic region. For example, the Western Pacific clade consisted of *N. pompilius* specimens collected in Fiji and American Samoa along with a specimen of *N. macromphalus* collected in New Caledonia. Wray *et al.* (1995) concluded that *N. belauensis*, *N. macromphalus*, and *N. stenomphalus* were morphological variants of a broadly distributed *N. pompilius*. While this study suggested the existence of only two phylogenetic species, it was also consistent with the hypothesis that present-day nautilus are currently undergoing a period of diversification (Saunders 1981b, 1987, Ward 1984, Teichert and Matsumoto 1987, Woodruff *et al.* 1987).

Bonnaud *et al.* (2004) observed substantial differences between the 18S rRNA sequences of *Allonautilus scrobiculatus* and those of *Nautilus macromphalus* and *N. pompilius*, supporting the validity of *Allonautilus*. There was very little sequence divergence between *N. pompilius* and *N. macromphalus*, and the authors agreed with Wray *et al.* (1995) that the distinctive shell of *N. macromphalus* was most likely a geographic variant of *N. pompilius*.

These studies clearly suggest that *Allonautilus scrobiculatus* represents an evolutionary lineage that is separate from the genus *Nautilus*. However, the number of species in the genus *Nautilus* remains unclear, as does the taxonomic status of *N. pompilius*. In addition, previously published phylogenetic studies either did not include an outgroup (Wray *et al.* 1995) or were unable to resolve the phylogenetic relationships within the genus *Nautilus* (Ward and Saunders 1997).

We address the issue of speciation in *Nautilus* and *Allonautilus* using data for another mitochondrial gene region, Cytochrome Oxidase *c* subunit I (COI), for all of the taxa included in the Wray *et al.* (1995) study, as well as an additional 48 specimens representing *N. pompilius* collected from seven different localities in the Indo-Pacific, and *N. macromphalus* collected from two different localities in New Caledonia (Fig. 2). This gene region was selected for its utility as a source of characters for phylogenetic analyses (Remigio and Hebert 2003) due to its tendency to show low levels of intraspecific variation and high levels of interspecific variation. In addition, this particular gene region has recently attracted a great deal of attention due to its use in molecular barcoding studies (Hebert *et al.* 2003a, 2003b, Ratnasingham and Hebert 2007). We collected these data, as well as additional 16S rDNA (16S) sequence data, for all of the additional samples and then analyzed the combined and individual molecular data sets using maximum parsimony. We also conducted a Population Aggregation Analysis (PAA) (Davis and Nixon 1992) to identify suites of diagnostic molecular characters for individual populations and species and analyzed the biogeographic distributions of the various species and populations included in the study.

We address the following questions. What is the relationship of *Allonautilus* to other present-day nautilus? Are *Nautilus macromphalus, N. stenomphalus, N. belauensis,* and *N. repertus* distinct phylogenetic species or are they morphological variants of *N. pompilius*? Is *N. pompilius* a single widespread morphologically variable species, or is it a paraphyletic assemblage consisting of populations that belong to different evolutionary lineages? Can the biogeographic distribution of nautilus populations be related to their evolutionary history? And, lastly, do the phylogeny and ecology of present-day nautilus suggest a possible explanation for the patterns of extinction and radiation seen in the fossil record of the nautilids?

MATERIALS AND METHODS

The specimens used in this study were collected over a period of 20 years by several individuals. At the time of collection the specimens were either frozen or preserved in 70% ethanol. Tissue samples for the specimens collected in New Caledonia and Vanuatu have been deposited in the Invertebrate Zoology collections at the American Museum of Natural History. The associated DNA samples extracted from these specimens have been archived in the Ambrose Monell Cryo Collection at the American Museum of Natural History and at the University of Illinois Springfield. All other tissue and associated DNA samples are archived at the University of Illinois Springfield. Detailed collection information is provided in the Appendices along with the AMNH and UIS sample numbers and the GenBank Accession numbers for the corresponding sequences.

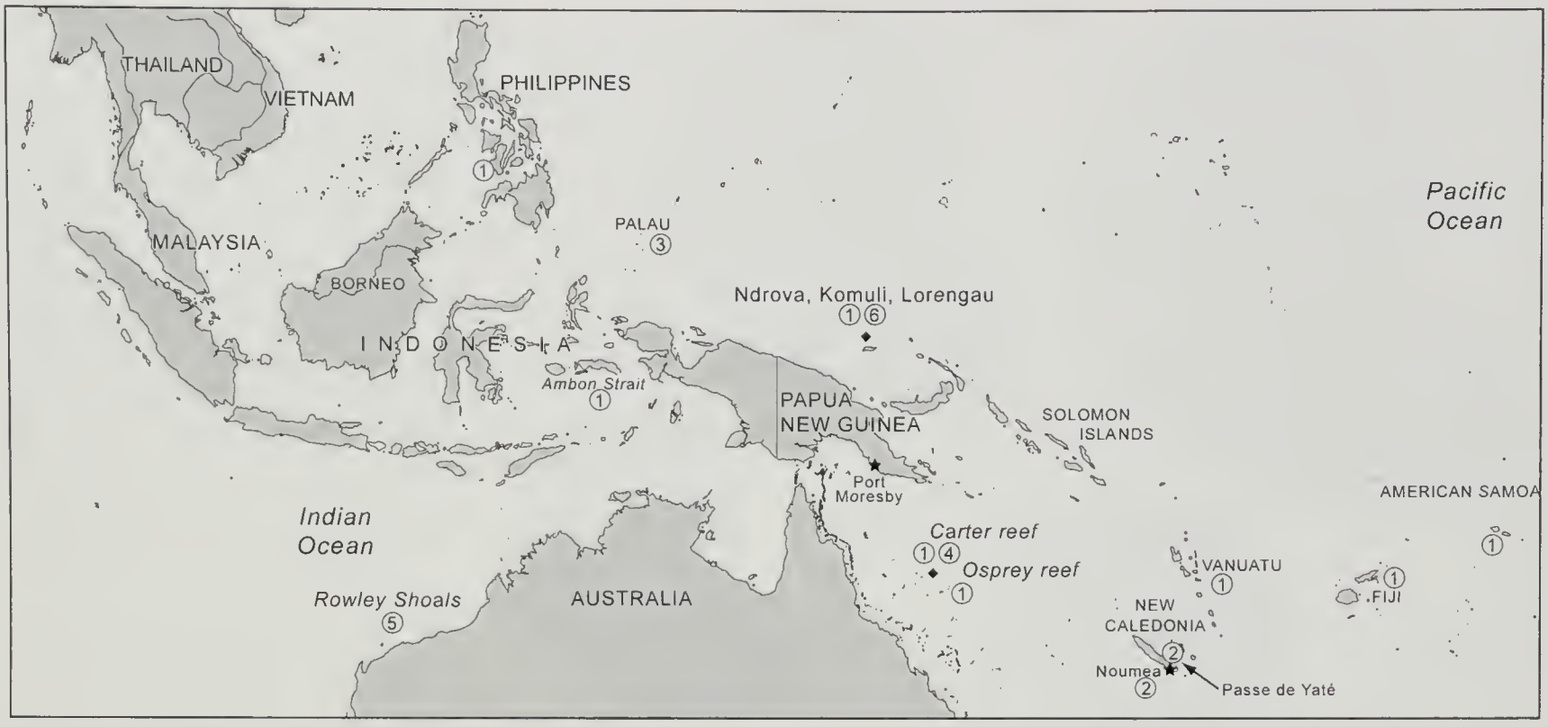

Figure 2. Distribution of nautilus populations included in this study. ①, *N. pompilius*; ②, *N. macromphalus*; ③, *N. belauensis*; ④, *N. stenomphalus*; ⑤, *N. repertus*; ⑥, *A. scrobiculatus*. Sites where individuals of two species occur sympatrically are indicated with a "♦". For collection information see Table 1.

DNA extraction, amplification and sequencing

Extraction of DNA was carried out on either mantle or jaw muscles using the DNAEasy kit (Qiagen) according to the manufacturer's instructions. Extracts were stored at -20 °C for short-term usage. A 412 base pair (bp) region of the Cytochrome Oxidase c subunit I (COI) gene, corresponding to bases 160-572 in the published genome of *Nautilus macromphalus* (Boore 2006, GenBank NC007980), was amplified by the Polymerase Chain Reaction (PCR) using the HCO and LCO primers of Folmer *et al.* (1994). The 399 bp fragment of the 16S rDNA gene, corresponding to bases 2,836 to 3,236 in the *N. macromphalus* genome, was amplified using the 16sa and 16sb primers (Palumbi 1996). Although the original amplicons were larger for both fragments, the actual data sets used for the various analyses are somewhat shorter. This is because the length of readable sequence varied among the different fragments. If we had used the complete sequences, we would have had to include a substantial amount of missing data. Consequently, we trimmed the ends of the sequences to a standard length in order to obtain the largest possible data set that included all characters for each of the samples. Standard reagents were used, and the reactions were carried out in a volume of 25 µl. Amplification conditions for both gene regions were 5 minutes at 94 °C, followed by 40 cycles of 30 seconds at 94 °C, 30 seconds at 46 °C, and 30 seconds at 72 °C, with a final extension step of 7 minutes at 72 °C. PCR products were visualized by agarose gel electrophoresis, and amplification products were purified using AMPure beads (Agencourt Corp.) according to the manufacturer's instructions. Sequencing reactions were performed in both directions with the amplification primers using BigDye v1.1 (Applied Biosystems) according to the manufacturer's instructions. Cycle sequencing products were precipitated with isopropanol, cleaned with 70% ethanol, resuspended in formamide, and read by an ABI Prism 377 DNA Sequencer. Sequence reads from the 5' and 3' directions were assembled into contigs and edited using the computer program Sequencher v4.6 (Gene Codes Corporation). The edited sequences were combined with previously published 16S sequences from Wray *et al.* (1995) and aligned using Sequencher 4.6. In several instances, this gene region was re-sequenced from new DNA extracts prepared from the same specimens to clarify ambiguities in the published sequences. The alignment was verified by visual inspection. Alignments were trivial, and only a single indel occurs within the 16S sequence. The gastropod species *Crepidula striolata* (Menke, 1851) was included as an outgroup to root the phylogeny. The GenBank accession numbers for the *Crepidula* sequences were AF545972.1 (16S) and AF353123.2 (COI).

Phylogenetic analysis

The aligned sequences were exported from Sequencher 4.6 as nexus files. The 16S and COI files were analyzed individually and also combined for the purpose of performing

a simultaneous analysis (SA) (Nixon and Carpenter 1996) using PAUP v.4.0b3 (Swofford 2003). Due to the large number of taxa included in the analysis, the heuristic search algorithm was employed with the tree bisection reconnection (TBR) branch swapping option. Maximum parsimony was chosen as the optimality criterion. One hundred random stepwise additions were performed. PAUP also was used to calculate bootstrap values (BV) for the entire data matrix by performing 1000 bootstrap replicates with 10 random additions of taxa per replicate. Partitioned Bremer support values (BS; Bremer 1992, Baker *et al.* 1998) were calculated with the computer program TreeRot v2b (Sorenson 1999) using the strict consensus tree recovered from the SA. Prior to performing the bootstrap and Bremer support analyses, all redundant taxa (individuals with identical haplotypes) were deleted from the analysis with the exception of the two representatives of the Fiji population. Both of these individuals were included in order to determine bootstrap and Bremer support values for this population.

Estimation of genetic distances

Uncorrected p distances were calculated for the SA data matrix using PAUP v.4.0b3 (Swofford 2003). All pair wise comparisons between individuals included in the ingroup were performed. The pair wise distances were exported into an Excel® (ver. 10) spreadsheet and average genetic distances for various subsets of the data were calculated using the descriptive statistics function in the Data Analysis tool pack.

We calculated the average genetic distance of all pair wise comparisons within both *Nautilus* and *Allonautilus,* as well as the average genetic distance from all pair wise comparisons between individuals in the two genera. We also calculated the average genetic distance for all pair wise comparisons of individuals within the Western Australia/Indonesia, Australia/Papua New Guinea, and Western Pacific clades (as defined by the results of the phylogenetic analysis). The average genetic distances among the Western Australia/Indonesia, Australia/Papua New Guinea, and Western Pacific clades were calculated by averaging the results of all pair wise comparisons of individuals in one clade with all individuals in another clade (*e.g.*, all pair wise comparisons between individuals in the Western Australia/Indonesia clade and individuals in the Australia/Papua New Guinea clade). The average genetic distance between *Allonautilus* and each of the three biogeographic clades was calculated by taking the average of all pair wise comparisons between the three representatives of *Allonautilus* included in the study and the individuals within each of the three clades. Lastly, the average genetic distance between one of the samples collected at Lorengau, and the individuals in each of the three biogeographic clades was calculated as well as the average genetic distance between this sample and the three samples of *Allonautilus.* This particular sample was selected because Wray *et al.* (1995: 225) noted that it possessed an "unusual mt haplotype" and formed a trichotomy with the Western Pacific and Australia/Papua New Guinea clades in the results of their analysis.

Population aggregation analysis

In addition to the 67 taxa included in the SA, 25 additional 16S sequences and 60 additional COI sequences were obtained from specimens from the Ndrova and Vanuatu populations of *Nautilus pompilius*, and from individuals of *N. macromphalus* collected at two different localities in New Caledonia, Noumea, and Passe de Yaté. These individuals were not included in the SA because each was represented by only one of the two data partitions included in the SA, and their inclusion in the phylogenetic analysis would have resulted in the introduction of a substantial amount of missing data.

These additional sequences were combined with the appropriate gene regions used in the SA to perform a Population Aggregation Analysis (PAA) (Davis and Nixon 1992). This is a non-tree based approach that identifies species on the basis of diagnostic characters. Two types of characters are considered diagnostic. A pure diagnostic character is defined as one where a particular character state is fixed in a particular population or group of populations but does not occur in any other populations. Private diagnostic characters are defined by the presence of a unique character state that occurs in some, but not all individuals, in a particular population or group of populations, but do not occur in any other populations. We included the additional sequences in this analysis because the larger sample sizes provided a more rigorous test of the diagnostic abilities of the individual characters.

The PAA was performed by a visual inspection of the individual data matrices using MacClade v4.0 (Maddison and Maddison 2000). The locations and numbers of pure and private diagnostic characters (Davis and Nixon 1992) for each gene region were determined for each population, or species that was represented by more than a single individual. In the case of populations or species that were represented by a single individual (*e.g.*, *Nautilus stenomphalus*), autapomorphies were scored as private diagnostic characters.

Biogeographic analysis

In order to reconstruct the biogeographic history of the populations in the study, all 14 collection localities were coded as an unordered multistate character. The ancestral states for this character were reconstructed for each node in the strict consensus of the maximum parsimony trees under the criterion of maximum parsimony using the computer program Mesquite (Maddison and Maddison 2004).

RESULTS

Phylogenetic analysis

The data matrix consisted of 811 molecular characters. Within the ingroup, 648 characters were constant, 43 characters were parsimony uninformative, and 120 characters were parsimony informative (PI). The 16S data partition contributed 40 of the PI characters, and the COI data partition contributed 80.

The SA produced 24 most parsimonious trees (MPTs), 564 steps in length with a consistency index (CI) of 0.822, and a retention index (RI) of 0.938. The strict consensus of these trees is shown (Fig. 3). Our results indicate that *Allonautilus scrobiculatus* is the sister taxon to all other proposed species within *Nautilus*, and that the various populations/species sampled within *Nautilus* form the same three distinct clades associated with their biogeographic distribution that were originally described by Wray *et al.* (1995). Following their nomenclature, the Western Pacific clade (Node III in Fig. 3) consists of *Nautilus macromphalus* and samples of *N. pompilius* collected in Vanuatu, Fiji, and American Samoa. The Australia/Papua New Guinea clade (Node VI in Fig. 3) includes samples of *N. stenomphalus*, *N. pompilius*, and a specimen identified by its collectors as a hybrid of *N. stenomphalus* and *N. pompilius* that was collected at Carter Reef (identified as Lizard Island in Wray *et al.* 1995), as well as specimens of *N. pompilius* that were collected at Osprey Reef. Both of these collection sites are located on the Great Barrier Reef in Queensland. This clade also contains specimens from several localities in Papua New Guinea (PNG). These are Ndrova Island, Komuli Island, and Lorengau, which are located within the province of Manus (PNG), and Port Moresby, which is on the southern coast of the island of New Guinea. The Western Australia/Indonesia clade (Node V in Fig. 3) includes a single specimen of *N. belauensis*, two specimens of *N. repertus*, two specimens of *N. pompilius* from the Ambon Strait in Indonesia, and three specimens of *N. pompilius* that were collected in the Philippines.

The individual analyses of the 16S and COI data partitions recovered all monophyletic groups that were observed in the SA (data not shown) with the exception of the Western Australia/Indonesia clade, which was not recovered by the individual analysis of the 16S data partition. The results of the individual analyses both indicate that *Allonautilus* is the sister taxon to *Nautilus*, but neither the 16S or COI data partitions alone were able to resolve the basal relationship among the three biogeographic clades within *Nautilus*. In the individual analysis of the 16S data partition a sample collected at Lorengau that Wray *et al.* (1995) described as having a unique haplotype, was not placed within any of the three biogeographic clades, which is consistent with the results they obtained. In contrast, the individual analysis of the COI data places this specimen (asterisk in Fig. 3) at the base of the Australia/Papua New Guinea clade.

All of the placements in the SA are consistent with the results of Wray *et al.* (1995) in that we also recovered the same three biogeographic clades that they first described, and in our results the placement of individuals and specific populations are consistent with their geographic distribution. But unlike the previous study (Wray *et al.* 1995) which recovered only two phylogenetic species (*Nautilus pompilius* and *Allonautilus scrobiculatus*), the more extensive sampling and additional data in our study indicate that the genus *Nautilus* is not monospecific as previously thought. Our results indicate that *N. macromphalus* is not a morphological variant of *N. pompilius*, but a distinct phylogenetic species. Furthermore, our results indicate that *N. pompilius* is not a single broadly distributed species but a paraphyletic assemblage of populations inhabiting many different reef systems throughout the Indo-Pacific. Several of these populations form monophyletic groups in both the SA and individual analyses, suggesting that they are phylogenetic species.

The Western Pacific clade is sister to a monophyletic group consisting of the other two biogeographic clades. Within the Western Pacific clade, *Nautilus macromphalus* forms a monophyletic group. Two populations of *N. macromphalus* were sampled, one from the southwest coast of New Caledonia, near Noumea, and the other from the southeastern side of the island near the town of Yaté. Together, the samples from both populations form a single well-supported monophyletic group (BV=100; BS=13). This is not surprising as both populations are located on the reef system surrounding the island of New Caledonia and there are no barriers to migration between the two localities. The presence of identical haplotypes in both populations suggests that *N. macromphalus* represents all of the individual nautilids inhabiting the reefs surrounding the island of New Caledonia and that together these individuals comprise a single panmictic population.

Nautilus macromphalus is the sister group to a clade that contains three populations of *N. pompilius*. Two of these populations, those from Vanuatu and Fiji, are monophyletic and possess fixed diagnostic characters and exclusive haplotypes, although the sampling of the Fiji population is not as extensive as that of the population in Vanuatu. The Vanuatu clade is well supported (BV=97: BS=4), as is the Fiji clade (BV=100; BS=11). The population in American Samoa is represented by a single individual, which is placed outside of the other populations in the Western Pacific clade.

In the Western Australia/Indonesia clade, the three *Nautilus pompilius* samples from the Philippines form a monophyletic group that is sister to a clade that contains *N. pompilius* from the Ambon Strait in Indonesia, *N. repertus* from the west coast of Australia, and *N. belauensis* from Palau.

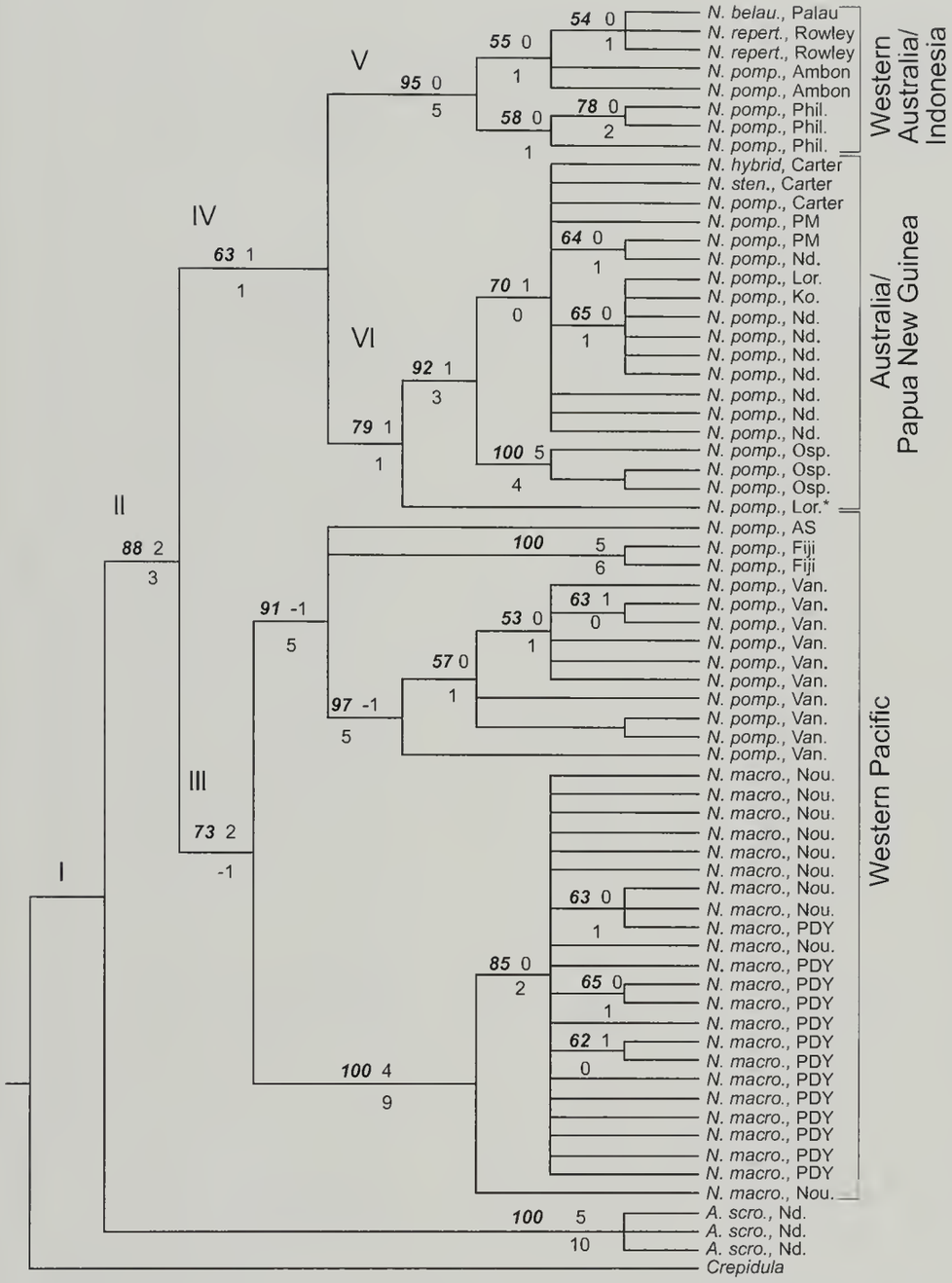

Figure 3. Strict Consensus of 24 most parsimonious trees recovered by the Simultaneous Analysis of the 16S and COI data. (Tree length = 564 steps, Consistency Index = 0.822, Retention Index = 0.938). Numbers in bold text above the branches at nodes represent bootstrap values. Numbers in plain text above the branches at nodes represent the Bremer Support contributed by the 16S data partition. Numbers in plain text below the branches at nodes represent the Bremer support contributed by the COI data partition. The sum of the Bremer support contributed by the individual data partitions at each node is the total Bremer support at that node. Roman numerals refer to specific nodes discussed in the text. (Abbreviations: AS, American Samoa; Carter, Carter Reef, Queensland, Australia; Ko, Komuli Island, PNG; Lor, Lorengau, Manus, PNG; Nd, Little Ndrova Island, PNG; Nou, Noumea, New Caledonia; Osp, Osprey Reef, Queensland, Australia; PDY, Passe de Yaté, New Caledonia; Phil, Philippines; PM, Port Moresby, PNG; Rowley, Rowley Shoals, W. Australia; Van, Vanuatu).

Support for the Philippine clade is weak (BV=58; BS=1) and is provided entirely by the COI data. Note that the two *N. repertus* samples form a weakly supported (BV=54; BS=1) polytomy with the single *N. belauensis* sample.

The Australia/Papua New Guinea clade contains three groups. There is a single well supported monophyletic group (BV=100; BS=9) consisting of 3 samples collected at Osprey Reef in Queensland. This population is the sister group to a larger clade consisting of samples collected at various sites in and around the island of New Guinea and Carter Reef. The Carter Reef specimens purportedly represent two species of *Nautilus*, which have been reported to occur sympatrically at this location (*N. pompilius* and *N. stenomphalus*), and an individual that was identified as a hybrid of the two. At the base of the entire Australia/Papua New Guinea clade is the individual collected at Lorengau, on Manus Island, Papua New Guinea, that formed a trichotomy with the Western Australia/Indonesia and Australia/Papua New Guinea clades in the results obtained by Wray *et al.* (1995). Support for this placement and the entire Australia/Papua New Guinea clade is relatively weak (BV=79; BS=2). Support for the node that includes the rest of the individuals assigned to the Australia/Papua New Guinea clade is high (BV=92; BS=4).

Genetic distance estimates

The average of the genetic distances between the various populations are reported (Table 1). The average genetic distance between individuals within the Western Pacific clade was 3%, which is three times higher than the average distances between the individuals in the other two biogeographic clades (1%). Also, the average genetic distance between the Western Pacific clade and the other two biogeographic clades (6% in both cases) was higher than the average genetic distance between the Western Australia/Indonesia and Australia/Papua New Guinea clades (4%). The average genetic distance between

Table 1. Genetic distance data. The average genetic distances between populations and/or individuals collected at each of the localities included in the study expressed as uncorrected p distances.

	Nautilus belauensis	*Nautilus repertus*	*Nautilus pompilius* Ambon Strait, Indonesia	*Nautilus pompilius* Philippines	*Nautilus pompilius* Komuli Island, Papua New Guinea	*Nautilus pompilius* Lorengau, Papua New Guinea	*Nautilus pompilius* Ndrova Island, Papua New Guinea
Nautilus belauensis	—						
Nautilus repertus	0.003	—					
Nautilus pompilius Ambon Strait	0.003	0.004	—				
Nautilus pompilius Philippines	0.010	0.012	0.008	—			
Nautilus pompilius Komuli Island, Papua New Guinea	0.036	0.040	0.036	0.038	—		
Nautilus pompilius Lorengau, Papua New Guinea	0.041	0.045	0.041	0.044	0.019	—	
Nautilus pompilius Ndrova Island, Papua New Guinea	0.035	0.039	0.035	0.036	0.003	0.018	—
Nautilus pompilius Osprey Reef, Queensland, Australia	0.042	0.045	0.042	0.042	0.020	0.032	0.018
Nautilus pompilius Port Moresby, Papua New Guinea	0.035	0.038	0.035	0.036	0.004	0.019	0.002
Nautilus stenomphalus	0.037	0.040	0.038	0.039	0.006	0.040	0.005
Nautilus macromphalus	0.059	0.063	0.057	0.061	0.058	0.063	0.057
Nautilus pompilius Vanuatu	0.056	0.060	0.057	0.059	0.059	0.063	0.058
Nautilus pompilius Fiji	0.063	0.062	0.061	0.066	0.063	0.068	0.062
Nautilus pompilius American Samoa	0.058	0.059	0.057	0.059	0.063	0.066	0.062
Allonautilus scrobiculatus	0.066	0.069	0.067	0.069	0.068	0.072	0.066

Table 1. (Continued)

Nautilus pompilius Osprey Reef, Queensland, Australia	*Nautilus pompilius* Port Moresby, Papua New Guinea	*Nautilus stenomphalus*	*Nautilus macromphalus*	*Nautilus pompilius* Vanuatu	*Nautilus pompilius* Fiji	*Nautilus pompilius* American Samoa	*Allonautilus scrobiculatus*
0.012	—						
0.021	0.005	—					
0.061	0.057	0.060	—				
0.059	0.058	0.061	0.052	—			
0.063	0.062	0.064	0.056	0.038	—		
0.063	0.062	0.064	0.056	0.035	0.037	—	
0.070	0.066	0.069	0.077	0.074	0.081	0.080	—

Allonautilus and the Western Pacific clade was 8% which was slightly higher than the 7% average genetic distance between the three representatives of *Allonautilus* and all 63 members of the genus *Nautilus* included in the study. The average genetic distance between *Allonautilus* and the other two biogeographic clades was 7% for both.

Wray *et al.* (1995: 225) reported that the sequence of one of the samples of *Nautilus pompilius* included in their study, that had been collected at Lorengau, in Papua New Guinea, had "numerous autapomorphic substitutions as well as synapomorphies with the other clades." We estimated the average genetic distance between this individual and each of the three biogeographic clades. Our results indicate that the average genetic distance between this individual and the other clades was 7% when it was compared with the Western Pacific clade, a distance as great as the average genetic distance between *Allonautilus* and *Nautilus*. The average genetic distance between this individual and the Western Australia/ Indonesia clade was 5%. Comparisons to the other individuals within the Australia/Papua New Guinea clade indicate the average genetic distance between this individual and the other members of the clade was 4%. This is almost as large as the average genetic distance between this individual and the Western Australia/Indonesia clade. It is also four times the average distance obtained from the other pairwise comparisons among individuals placed within the Australia/Papua New Guinea clade.

Population aggregation analysis

The results of the PAA are in agreement with the results of the SA and the individual analyses in that each of the monophyletic groups recovered by the phylogenetic analysis that corresponded to individual populations also possesses a suite of pure and private diagnostic characters (see Table 2). A total of 41 pure and 25 private diagnostic characters were recovered from both data partitions. The number of both types of diagnostic sites was slightly higher for the COI data partition, which accounted for 51% (21/41) of the pure and 54% (13/25) of the private diagnostic sites.

It was not possible to determine whether the *Nautilus pompilius* populations in American Samoa and Komuli Island, or whether *N. stenomphalus* and *N. belauensis* represent monophyletic groups because each is represented by a single individual. Autapomorphies in the sequences of these individuals therefore were counted as private diagnostic sites. The Komuli Island specimen and the single individual of *N. stenomphalus* each possess a single autapomorphy. The single individual representing the American Samoa population has 6 autapomorphies (3 in the 16S sequence and 3 in the COI sequence). All these autapomorphies may be pure or private diagnostic characters for these species or populations, but this cannot be determined without additional sampling of the populations. The *N. belauensis* sequences did not contain any autapomorphies, although they share a single pure diagnostic site with the two *N. repertus* samples, suggesting that these two putative species may be synonymous.

At the base of the Australia/Papua New Guinea clade is a specimen of *Nautilus pompilius* that was collected at Lorengau. This is the same specimen that was described as having a unique haplotype by Wray *et el.* (1995). The sequences contain 10 autapomorphies, 1 in the 16S sequence and 9 in the COI sequence.

Biogeographic analysis

The reconstruction of the ancestral character state at the nodes representing the divergence between *Allonautilus* and *Nautilus* (Node I in Fig. 3) indicates that this occurred in the waters off Papua New Guinea. This is also the case for the nodes representing the divergence events that led to the founding of each of the three clades within *Nautilus* (Nodes II and IV in Fig. 3), suggesting that the current radiation of present-day nautilus has its origin in this region. The reconstruction of the ancestral

Table 2. Results of the Population Aggregation Analysis.

		Number of diagnostic characters			
Clade	N COI	COI partition	N 16S	16S partition	Total
Allonautilus scrobiculatus	3	11	3	9	14
Nautilus macromphalus	83	9 (7 PRIVATE, 2 PURE)	38	5 (2 PRIVATE, 3 PURE)	14
Nautilus pompilius Vanuatu	13	5 (2 PRIVATE, 3 PURE)	14	8 (7 PRIVATE , 1 PURE)	13
Nautilus pompilius Fiji	2	4	2	3	7
Nautilus pompilius Osprey Reef	5	3 (1 PRIVATE, 2 PURE)	4	3	6
Nautilus pompilius Philippines	3	3 (PRIVATE)	3	3 (PRIVATE)	6

N COI and N 16S indicate the number of sequences for each species or population that was included in the Population Aggregation Analysis. Numbers in the COI partition and 16S partition columns indicate the number of diagnostic characters present in each data partition for each species or population. All characters are pure diagnostic characters (*sensu* Davis and Nixon 1992) unless otherwise indicated.

character states also indicates that the divergence represented by the basal node in the Australia/Papua New Guinea clade (Node VI) also occurred in Papua New Guinea.

The topology of the strict consensus tree indicates that the first divergence in *Nautilus* (Node II in Fig. 3) occurred when the common ancestor of the Western Pacific clade diverged from the common ancestor of the Australia/Papua New Guinea and Western Australia/Indonesia clades, presumably as a result of a migration to the east. The basal divergence within the Western Pacific clade (Node III in Fig. 3) occurred when *N. macromphalus* diverged from the common ancestor of the Fiji, Vanuatu, and American Samoa populations. If this occurred as a result of a migration from New Caledonia to Vanuatu followed by subsequent migrations to Fiji, and finally American Samoa, it would be consistent with a pattern of progressive dispersal to the east. The reconstruction of the ancestral character state at this node is equivocal though and we cannot at this time determine the order in which these populations were founded.

The migration to the east was followed by a second migration from Papua New Guinea, this time to the west by the ancestor of the Western Australia/Indonesia clade (represented by Node IV in Fig. 3). The ancestral character state reconstruction at the node representing the basal divergence within this clade (Node V in Fig. 3) is also equivocal, and we are unable to determine the order in which the four locations represented in this clade (Ambon Strait, Rowley Shoals, Palau, and the Philippines) were colonized.

DISCUSSION

Our results suggest that the present-day nautilids are undergoing a period of evolutionary radiation throughout the Indo-Pacific region. The ancestral character state reconstruction provided by the biogeographic analysis indicates that the divergence between *Allonautilus* and *Nautilus* (Node I in Fig. 3) occurred in the waters off Papua New Guinea. This is consistent with the observation that *A. scrobiculatus* and *N. pompilius* both occur at Ndrova Island (Saunders *et al.* 1987a). Also, the ancestral character state reconstructions at the nodes representing the migrations that led to the founding of the Western Pacific clade (Node II in Fig. 3), and its divergence from the common ancestor of the Western Australia/Indonesia and Australia/Papua New Guinea clades (Node IV in Fig. 3) indicate that these divergences also occurred in this region. Papua New Guinea lies at the geographic center of the distribution of all present day nautilus. For these reasons, we hypothesize that the waters off Papua New Guinea provided a refuge for the survivors of the events that led to the sudden decline of nautilids in the fossil record following the end of the Miocene.

The fact that samples collected in the western Pacific and western Australia/Indonesia regions form monophyletic groups in the topology of the strict consensus tree suggests that the evolutionary radiations in these regions may have occurred following a single founder event. While well adapted to life on sloping reef faces, present-day nautilus are rarely found in the open ocean. Saunders *et al.* (1987b) observed damage to the shell, mantle, and hood of trapped specimens that resulted from predation by octopus and fish. These authors reported direct observations of attacks on nautilus by triggerfish and groupers. The specimens that were attacked in these instances made no attempts to defend themselves beyond retracting into their shells, and nautilus in the water column appear to be easy prey for individuals of a variety of species. Attempts to avoid predation by seeking shelter along the ocean bottom are limited by cooler temperatures at depth, as well as the shell implosion depth of 600 m (Saunders and Ward 1987b). All present-day nautilus are also extremely slow swimmers, usually covering less than 2 km per day (Ward *et al.* 1984, Saunders and Ward 1987b). In order to travel significant distances across deep water basins, nautilus most likely require some form of passive transport such as an ocean current.

These limitations suggest that successful long distance migrations by nautilus occur infrequently and that, once new populations are established, gene flow between the new population and its source rarely occurs. This would lead to reproductive isolation of the new population. Our results indicate that individual populations that are separated from others by large expanses of deep water, for example in the Western Pacific clade or the Philippines, are monophyletic. In contrast, populations that are separated by shorter distances and shallower water, such as the two populations of *Nautilus macromphalus,* still show evidence of gene flow between populations.

The lack of monophyletic populations in the Australia/Papua New Guinea clade, with the exception of the one at Osprey Reef, provides another example of gene flow occurring among populations that are separated by small distances and shallow water. Swan and Saunders (1987), in a study of morphological variation among six populations of nautilus in and around Papua New Guinea, detected variation suggesting adaptation to local conditions, but the authors were unable to find any fixed variants in any of the populations they sampled. They suggested that gene flow was occurring among the populations in this region. The placement of the Port Moresby, Komuli, and Lorengau samples in the same clade in our analysis is in agreement with this, as is the lack of diagnostic molecular characters for any of the individual populations. Komuli and Ndrova are islands located just to the east of the larger island of Manus where the port city of Lorengau is located. These three locations all lie within 50 km of one another, and the waters separating Komuli and Ndrova

from Manus are less than 200 m deep, providing ample opportunity for gene flow to occur. Gene flow between the populations in Manus Island and the rest of Papua New Guinea would require migrations over deeper water, but the distance from Manus to the main island of New Guinea is approx. 350 km, considerably less than the distances that separate populations in the Western Pacific clade; the latter range from 600 to 700 km. However, direct contact among these populations may not be required. Reports of live nautilus from the reefs surrounding the islands of New Britain and New Ireland (House 1987) indicate that resident populations in these areas could provide an avenue for gene flow between the populations in Manus Island and those off the main island of New Guinea.

The inclusion of the Carter Reef samples (Great Barrier Reef) within the Australia/Papua New Guinea clade may seem surprising because a distance of 550 km separates these two locations. But the island of New Guinea is connected to the Australian mainland by the continental shelf and the depth of the water separating these locations is 200 m or less, suggesting that gene flow could occur between these locations. This implies that gene flow can occur between individual populations of nautilus from Lorengau on Manus Island in the north to the island of New Guinea, and even further south to the Great Barrier Reef across a distance of 1200 km. Woodruff *et al.* (1987) previously suggested this possibility based on an analysis of allozyme data.

The samples collected at Osprey Reef form the only monophyletic group within the Australia/Papua New Guinea clade. Osprey Reef is separated from the continental shelf by approx. 160 km across the Queensland trough, where water depths exceed 1000 m. This is less than the presumed distance that migrants would have to travel between Manus Island and New Ireland, the two closest reef systems that are separated by deep water, where specimens of nautilus are known to occur. At Osprey Reef, however, there appears to have been a successful barrier to dispersal. Osprey Reef is a comparatively small island, only 25 km in length, compared to the 325 km length of New Ireland. Consequently, Osprey Reef is a much smaller target for an animal drifting in an ocean current to reach by chance, and the nautilus population at Osprey Reef may have been established by a single colonization event.

A single individual identified as *Nautilus stenomphalus* is placed within the Australia/Papua New Guinea clade. It was collected at Carter Reef, along with a specimen identified as *N. pompilus* and a putative hybrid of these two species. The relationship of these three specimens to one another as well as to the other individuals within this clade remains unresolved.

At the base of the Australia/Papua New Guinea clade is the individual collected at Lorengau that was identified as having a unique haplotype by Wray *et al.* (1995). It is sister to all other individuals within this clade, and the sequence divergence between this individual and all other members of the Australia/Papua New Guinea clade is 4%. In contrast, the average uncorrected p distance of all pairwise comparisons between the other individuals within this clade is 1%. Wray *et al.* (1995) speculated that it might be a migrant from a different reef system. If this is so, its source population represents a lineage that originated as a result of the earliest divergence within the Australia/Papua New Guinea clade surveyed to date, and its migration to Lorengau suggests that gene flow can occur between these populations. Additional sampling of nautilus in this area is called for before this can be determined, but at the very least it suggests that there is much more to be learned about the biogeographic history of nautilus populations.

In the Western Australia/Indonesia clade, the three *Nautilus pompilius* specimens from the Philippines form a monophyletic group that is sister to a clade that contains *N. pompilius* from the Ambon Strait in Indonesia, *N. repertus* from the west coast of Australia, and *N. belauensis* from Palau. The two *N. repertus* samples form a weakly supported (BV=54; BS=1) polytomy with the single *N. belauensis* individual. Both of these putative species are characterized as having the largest shells within the genus, and both the 16S and COI sequences for these three individuals are extremely similar. Only two sites within the COI data distinguish one of the *N. repertus* specimens from the other two species in this clade, suggesting the possibility that *N. belauensis* may be synonymous with *N. repertus*. This is somewhat surprising in light of the fact that Rowley Shoals, the collection locality for the two *N. repertus* specimens, is over 3000 km from Palau, where *N. belauensis* occurs. These sequences are also very similar to those of the Ambon Strait samples and the Philippine samples, suggesting that diversification within the Western Australia/Indonesia clade is either occurring very slowly or has begun more recently relative to the Western Pacific clade.

The fact that we did not include morphological characters in our study should not be misinterpreted to mean that we do not recognize the importance of these data. The authors of previous studies who utilized morphological data were unable to resolve phylogenetic relationships of nautilus beyond differentiating between *Allonautilus* and *Nautilus* (Wray *et al.* 1995, Ward and Saunders 1997). Of the ten morphological characters analyzed by Wray *et al.* (1995), all were phylogenetically uninformative although six of them were diagnostic for *A. scrobiculatus*. Ward and Saunders (1997) expanded on this study and analyzed a series of 20 morphological characters for *N. pompilius*, *N. macromphalus*, *N. belauensis*, *A. scrobiculatus*, and *A. perforatus*, as well as eight fossil nautilids. None of these characters resolved the phylogenetic relationships of the extant species although a subset of these characters served to separate *A. scrobiculatus*

and *A. perforatus* from *N. macromphalus*, *N. belauensis*, and *N. pompilius*, and two characters were diagnostic for *N. stenomphalus*. We recognize that a detailed comparative study of the morphology of different populations is called for to assess patterns of morphological variation.

The lack of phylogenetic information contained in the morphological characters analyzed to date may be due to a genuine lack of variation indicative of a series of recent divergences, or the fact that these characters have yet to be explored in sufficient detail. At the present time, the available morphological characters are unable to resolve the phylogeny of *Nautilus* and *Allonautilus*. The identification of phylogenetically informative morphological characters in present-day nautilus remains one of the greatest challenges in the study of these animals.

Our results indicate that *Allonautilus* is a sister taxon to *Nautilus*. This is in agreement with Wray *et al.* (1995) and Ward and Saunders (1997), and we concur that *Allonautilus* should be recognized as a separate genus within the Nautilidae. Also, *N. macromphalus* is an independent evolutionary lineage within *Nautilus* and is, therefore, a valid phylogenetic species. In contrast, *N. pompilius* is a paraphyletic assemblage of nautilus populations found throughout the Indo-Pacific. The phylogenetic hypothesis represented by the strict consensus tree we recovered from our data indicates that the nautilus populations in Vanuatu, Fiji, the Philippines, and Osprey Reef are all independent, reproductively isolated evolutionary lineages and, therefore, represent distinct phylogenetic species. Although the population at American Samoa is represented by a single individual, the large number of autapomorphies it possesses, as well as its geographic isolation, suggests that this population also may represent a phylogenetic species.

The fact that different populations of *Nautilus pompilius* may represent distinct phylogenetic species has been suggested by previous studies. For example, a significant degree of genetic differentiation between populations of *N. pompilius* in the Philippines and in Fiji was reported by Masuda and Shinomiya (1983). A comparison of biomineralization characteristics of specimens from these two populations indicated differences in magnitude as great as those attributed to interspecific differences between individual representatives of *N. pompilius*, *N. macromphalus*, and *Allonautilus scrobiculatus* (Crick and Mann 1987). The allozyme study of Woodruff *et al.* (1987) showed that the genetic distance separating individuals of *N. pompilius* collected in Fiji from supposedly conspecific individuals from localities in Papua New Guinea is sufficient to assign them to separate species. Wray *et al.* (1995), despite the fact that they were able to distinguish only two phylogenetic species due to the limited sampling in their study, also considered this possibility. We conclude that *N. pompilius*, over the years, has become a "catch all taxon" to which all specimens lacking a distinctive morphological difference (*e.g.*, the open umbilicus of *N. macromphalus*) have been assigned. This underscores the need for a more detailed study of nautilus morphology, which may reveal subtle differences between individual populations identified as *N. pompilius*.

The taxonomic status of *Nautilus belauensis*, *N. stenomphalus*, and *N. repertus* remains unclear at this time. Our results suggest that *N. belauensis* and *N. repertus* may be synonymous, but more extensive sampling is called for before this determination can be made, especially in light of the large geographic distance that separates the localities where these individuals were collected. Although the distance between Palau and the west coast of Australia is vast, there are many small islands that lie between them, and the possibility that *N. repertus* and *N. belauensis* may maintain reproductive contact through intermediate populations within these islands awaits further exploration.

The topology of the strict consensus tree indicates that a population of present-day nautilus in the Papua New Guinea region may have been the source from which all present-day nautilus were derived. We hypothesize that speciation is occurring allopatrically as a result of a series of founder events. The monophyletic groups our study recovered that correspond to individual collection localities in Fiji, Vanuatu, New Caledonia, Osprey Reef, and the Philippines support this. Our results also indicate that the extant species of nautilus seem to be recovering from their decline at the beginning of the Miocene. This may be a direct result of the fact that their adaptation to life on reef faces limits their ability to disperse. This limitation on dispersal suggests that successful migrations rarely occur. But on the occasions when they do, these same limitations also lead to reproductive isolation, and the formation of new species. This presents one possible explanation for the alternating periods of diversification and decline seen in the fossil record of nautilids.

Many aspects of the evolution and biogeography of present-day nautilus await further study. Greater sampling of individual populations undoubtedly will help to develop a more detailed picture of the dispersal and evolution of present-day nautilus. Of particular interest is the phylogenetic placement of *Allonautilus perforatus*, which at the present time is known only from drift shells. This species, which Ward and Saunders (1997) assigned to *Allonautilus* on the basis of its shell morphology, is thought to inhabit the reefs in the vicinity of Bali, Indonesia. If it is indeed a member of the same lineage as *A. scrobiculatus*, the biogeographic and evolutionary history of present-day nautilus may have been much more complex than previously imagined. Finally, the incorporation of morphological data for present-day nautilus, based on a careful, exhaustive analysis, may well provide insights into the significance of the morphological features of

fossil nautilids, providing fresh perspectives on the systematics and phylogenetic history of this group.

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LITERATURE CITED

Baker, R. H., X. Yu, and R. DeSalle. 1998. Assessing the relative contributions of molecular and morphological characters in simultaneous analysis trees. *Molecular Phylogenetics and Evolution* **9**: 427-436.

Bonnaud, L., C. Ozouf-Costaz, and R. Boucher-Rodoni. 2004. A molecular and karyological approach to the taxonomy of *Nautilus*. *Comptes Rendus Biologiques* **327**: 133-138.

Boore, J. L. 2006. The complete sequence of the mitochondrial genome of *Nautilus macromphalus* (Mollusca: Cephalopoda). *BMC Genomics* **7**:182. Available at: http://www.biomedcentral.com/content/pdf/1471-2164-7-182.pdf.

Bremer, K. 1992. Branch support and tree stability. *Cladistics* **10**: 295-304.

Cracraft, J. 1983. Species concepts and speciation analysis. *Current Ornithology* **1**: 159-187.

Crick, R. E. and K. O. Mann. 1987. Biomineralization and systematic implications. *In*: W. B Saunders and N. H. Landman, eds., Nautilus*: The Biology and Paleobiology of a Living Fossil.* Topics in Geobiology 6. Plenum Press, New York. Pp. 115-134.

Davis, J. L. and K. C. Nixon. 1992. Populations, genetic variation and the delimitation of species. *Systematic Biology* **41**: 421-435.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294-299.

Harvey, A., R. Mooi, and T. M. Gosliner. 1999. Phylogenetic taxonomy and the status of *Allonautilus* Ward and Saunders. *Journal of Paleontology* **73**: 1214-1217.

Hebert, P. D. N., S. Ratnasingham, and J. R. deWaard. 2003a. Barcoding animal life: Cytochrome c oxidase subunit divergences among closely related species. *Proceedings of the Royal Society of London* (B, Biological Sciences) **270**: 596-599.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003b. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London* (B, Biological Sciences) **270**: 313-321.

House, M. R. 1987. Geographic distribution of *Nautilus* shells. *In:* W. B. Saunders and N. H. Landman, eds., Nautilus*: The Biology and Paleobiology of a Living Fossil.* Topics in Geobiology 6. Plenum Press, New York. Pp. 53-64.

Kummel, B. 1956. Post-Triassic nautiloid genera. *Bulletin of the Museum of Comparative Zoology* **114**: 324-494.

Maddison, D. R. and W. P. Maddison. 2000. *MacClade, Version 4.0.* Sinauer, Sunderland, Massachusetts.

Maddison, W. P. and D. R. Maddison. 2004. *Mesquite, Version 1.02.* http://mesquiteproject.org.

Masuda, Y. and A. Shinomiya. 1983. Genetic variation in *Nautilus pompilius*. *Occasional Papers of the Kagoshima University Research Center for the Pacific Islands.* Studies on *Nautilus pompilius* and its Associated Fauna from Tanon Strait, the Philippines **1**: 22-25.

Matsumoto, T. 1983. Cretaceous nautiloids from Hokkaido-I. *Transactions of the Proceedings of the Paleontological Society of Japan* (new series) **129**: 9-25.

Miller, A. K. 1947. Tertiary nautiloids of the Americas. *Geological Society of America Memoir* **23**: 1-234.

Nixon, K. C. and J. M. Carpenter. 1996. On simultaneous analysis. *Cladistics* **12**: 221-241.

Palumbi, S. R. 1996. Nucleic acids II: the polymerase chain reaction. *In:* D. M. Hillis, C. Moritz, and B. K. Mable, eds., *Molecular Systematics.* Sinauer Associates, Sunderland, Massachusetts. Pp. 205-247.

Ratnasigham, S. and P. D. N. Hebert. 2007. BOLD: The barcoding of life data system. *Molecular Ecology Notes* **7**: 355-364.

Remigio, E. A. and P. D. N. Hebert. 2003. Testing the utility of partial COI sequences for phylogenetic estimates of gastropod relationships. *Molecular Phylogenetics and Evolution* **29**: 641-647.

Saunders, W. B. 1981a. A new species of *Nautilus* from Palau. *The Veliger* **24**: 1-7.

Saunders, W. B. 1981b. The species of *Nautilus* and their distribution. *The Veliger* **24**: 8-17.

Saunders, W. B. 1987. The species of *Nautilus. In:* W. B. Saunders and N. H. Landman, eds., Nautilus*: the Biology and Paleobiology of a Living Fossil.* Topics in Geobiology 6. Plenum Press, New York. Pp. 35-52.

Saunders, W. B. and C. Spinosa. 1979. *Nautilus* movement and distribution in Palau, Western Caroline Islands. *Science* **294**: 1199-1201.

Saunders, W. B. and P. D. Ward. 1987a. Sympatric occurrence of living *Nautilus* (*N. pompilius* and *N. stenomphalus*) on the Great Barrier Reef, Australia. *The Nautilus* **101**: 188-193.

Saunders, W. B. and P. D. Ward. 1987b. Ecology, distribution and population characteristics of *Nautilus. In:* W. B Saunders and N. H. Landman, eds., Nautilus*: the Biology and Paleobiology of a Living Fossil.* Topics in Geobiology 6. Plenum Press, New York. Pp. 137-162.

Saunders, W. B., L. E. Davis, and R. L. Knight. 1987a. Sympatric species of *Nautilus* (*N. pompilius* and *N. scrobiculatus*) in the Admiralty Islands, Papua New Guinea. *The Nautilus* **101**: 93-99.

Saunders, W. B., C. Spinosa, and L. E. Davis. 1987b. Predation on *Nautilus*. *In*: W. B Saunders and N. H. Landman, eds., Nautilus: *The Biology and Paleobiology of a Living Fossil*. Topics in Geobiology 6. Plenum Press, New York. Pp. 201-214.

Saunders, W. B., V. N. Shimansky, and O. V. Amitrov. 1996. Clarification of *Nautilus praepompilius* from the Upper Eocene of Kazhakstan. *Journal of Paleontology* **70**: 609-611.

Sorenson, M. D. 1999. *Treerot, Version.2b*. Boston University, Boston, Massachusetts.

Swan, A. R. H. and W. B. Saunders. 1987. Morphological variation in *Nautilus* from Papua New Guinea. *In:* W. B. Saunders and N. H. Landman, eds., Nautilus*: the Biology and Paleobiology of a Living Fossil*. Topics in Geobiology 6. Plenum Press, New York. Pp. 85-104.

Swofford, D. 2003. *PAUP: Phylogenic Analysis Using Parsimony, Version 4.0b1*. Sinauer Associates, Sunderland, Massachusetts.

Teichert, C. and T. Matsumoto. 1987. The ancestry of the genus *Nautilus*. *In*: W. B Saunders and N. H. Landman, eds., Nautilus*: the Biology and Paleobiology of a Living Fossil*. Topics in Geobiology 6. Plenum Press, New York. Pp. 25-32.

Tintant, H. and M. Kabamba. 1983. Le nautile, fossile vivant ou forme cryptogène? Essai sur l'evolution et la classification des Nautilaces. *Bulletin de la Société Zoologique de France*. **108**: 569-579 [In French].

Wani, R., R. De Ocampo, Y. Aguilar, M. Zepeda, Y. Kurihara, K. Hagino, H. Hayashi, and T. Kase. 2007. First discovery of fossil *Nautilus pompilius* Linnaeus, 1758 (Nautilidae, Cephalopoda) from Pangasinan, northwestern Philippines. *Paleontological Research* **12**: 89-95.

Ward, P. D. 1984. Is *Nautilus* a living fossil? *In:* N. Eldredge and S. Stanley, eds., *Living Fossils*. Springer, New York. Pp. 247-256.

Ward, P. D. 1988. *In Search of Nautilus*. Simon and Schuster, New York.

Ward, P. D. 1999. Phylogenetic taxonomy and the status of *Allonautilus* Ward and Saunders, 1997: Reply. *Journal of Paleontology* **73**: 1217.

Ward, P. D. and W. B. Saunders. 1997. *Allonautilus*: A new genus of living nautiloid cephalopod and its bearing on the phylogeny of the Nautilida. *Journal of Paleontology* **71**: 1054-1064.

Ward P., B. Carlson, M. Weekly, and B. Brumbaugh. 1984. Remote telemetry of daily vertical and horizontal movement of *Nautilus* in Palau. *Nature* **309**: 248-250.

Willey, A. 1899. General account of a zoological expedition to the South Seas during the years 1894-1897. *Proceedings of the Zoological Society of London* **1899**: 7-9.

Willey, A. 1902. Contribution to the natural history of the pearly *Nautilus*: Zoological results based on material from New Britain, New Guinea, Loyalty Islands and elsewhere, collected during the years 1895, 1896, and 1897: Part **6**: 691-830. University Press, Cambridge, UK.

Woodruff, D. S., M. P. Carpenter, W. B. Saunders, and P. Ward. 1987. Genetic variation and phylogeny in *Nautilus*. *In:* W. B. Saunders and N. H. Landman, eds., Nautilus*: the Biology and Paleobiology of a Living Fossil*. Topics in Geobiology 6. Plenum Press, New York. Pp. 65-83.

Wray, C. G., N. H. Landman, W. B. Saunders, and J. Bonacum. 1995. Genetic divergence and geographic diversification in *Nautilus*. *Paleobiology* **21**: 220-228.

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Appendix 1. Samples archived at the American Museum of Natural History.

Species	AMNH specimen #	GenBank accession #		Collection location	Collection date
		COI	16S		
Nautilus macromphalus	185260	GQ280217	GQ280151	Noumea, New Caledonia	October 2003
	185263	GQ280218	GQ280152		
	185264	GQ280219	GQ280153		
	185266	GQ280220	GQ280154		
	185267	GQ280221	GQ280155		
	185269	GQ280222	GQ280156		
	185270	GQ280223	GQ280157		
	185272	GQ280224	GQ280158		
	185273	GQ280225	GQ280159		
	185275	GQ280226	GQ280160		
	185360	GQ280227	GQ280161	Passe de Yaté, New Caledonia	January 2004
	185361	GQ280228	GQ280162		
	185362	GQ280229	GQ280163		
	185365	GQ280230	GQ280164		
	185368	GQ280231	GQ280165		
	185369	GQ280232	GQ280166		
	185373	GQ280233	GQ280167		
	185374	GQ280234	GQ280168		
	185381	GQ280235	GQ280169		
	185383	GQ280236	GQ280170		
	185384	GQ280237	GQ280171		
	185393	GQ280238	GQ280172		
	185407	GQ280239	GQ280173		
Nautilus pompilius	129515	GQ280240	GQ280174	Vanuatu	July 2004
	129517	GQ280241	GQ280175		
	129518	GQ280242	GQ280176		
	129519	GQ280243	GQ280177		
	129520	GQ280244	GQ280178		
	129521	GQ280245	GQ280179		
	129525	GQ280246	GQ280180		
	129526	GQ280247	GQ280181		
	129528	GQ280248	GQ280182		
	129530	GQ280249	GQ280183		

Appendix 2. Samples archived at the University of Illinois Springfield (UIS).

Species	UIS Sample identification #	GenBank accession #		Collection location	Collection date
		COI	16S		
Allonautilus scrobiculatus	427	GQ280250	GQ280184	Little Ndrova Island, Papua New Guinea	June 1984
	419	GQ280251	GQ280185		November 1984
	415	GQ280252	GQ280186		June 1985
Nautilus belauensis	425	GQ280187	U11625*	Palau	N/A
Nautilus repertus	423	GQ280188	GQ280124	Rowley Shoals, Western Australia	N/A
	424	GQ280189	GQ280125		
Nautilus stenomphalus	410	GQ280196	GQ280130	Carter Reef, Queensland, Australia	December 1985
Nautilus stenomphalus/pompilius hybrid	411	GQ280195	GQ280129	Carter Reef, Queensland, Australia	
Nautilus pompilius	409	GQ280197	GQ280131	Carter Reef, Queensland, Australia	
	207	GQ280198	GQ280132	Osprey Reef, Queensland, Australia	March 2003
	208	GQ280199	GQ280133		
	209	GQ280200	GQ280134		
	400	GQ280190	GQ280125	Ambon Strait, Indonesia	August 1984
	401	GQ280191	GQ280126		
	426	GQ280192	U11626*	Pangalao Island, Philippines	N/A
	701	GQ280193	GQ280127	Balayan Bay, Philippines	N/A
	702	GQ280194	GQ280128		
	420	GQ280201	GQ280135	Port Moresby, Papua New Guinea	October 1984
	421	GQ280202	GQ280136		
	22	GQ280206	GQ280140	Little Ndrova Island, Papua New Guinea	October 1984
	26	GQ280207	GQ280141		
	28	GQ280208	GQ280142		
	24	GQ280209	GQ280143		
	21	GQ280210	GQ280144		
	11	GQ280211	GQ280145		
	12	GQ280212	GQ280146		
	24	GQ280213	GQ280147		
	406	GQ280205	GQ280139	Komuli Island, Papua New Guinea	July 1984
	407	GQ280203	GQ280137	Lorengau, Manus Island, Papua New Guinea	July 1984
	408	GQ280204	GQ280138		
	402	GQ280214	GQ280148	Pago Pago, American Samoa	July 1984
	403	GQ280215	GQ280149	Suva, Fiji	August 1986
	404	GQ280216	GQ280150		

* Accession numbers for sequences used in this study and in Wray *et al.* (1995)